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Induction of protein synthesis in response to ultraviolet light, nalidixic acid and heat shock in the cyanobacterium *Phormidium laminosum*

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Synthesis of three polypeptides (43, 34 and 26 kDa) was enhanced or induced in the thermophilic cyanobacterium *Phormidium laminosum* following irradiation with UV light. Synthesis of several polypeptides was also enhanced/induced following treatment with nalidixic acid. Three had similar molecular masses to those induced by UV. One of these (43 kDa) was also apparently enhanced following prolonged heat shock, as were a number of other polypeptides with a wide range of molecular masses. The specific induction of the 34 and 26 kDa polypeptides following DNA damage and inhibition of replication suggests that they may be involved in DNA repair and, possibly, also in recombination.

DNA damage; Ultraviolet irradiation; Nalidixic acid; Heat shock; Protein synthesis; Cyanobacterium; (*Phormidium laminosum*)

1. INTRODUCTION

The processes of DNA repair and recombination in cyanobacteria are still very poorly characterised, although an understanding of them will be of great importance in genetic manipulation, and may in addition offer insights into repair and recombination in chloroplasts.

In many prokaryotes, DNA damage by irradiation with UV light has been shown to induce the synthesis of several proteins involved in DNA repair, some of which are also important in recombination. These include the RecA protein of *E. coli* [1], which is central to the 'SOS' regulatory network [2]. These responses can also be induced by agents that inhibit DNA synthesis, such as nalidixic acid and thymine starvation [3], although some of the proteins are also induced by heat shock [4] and may be part of a wider 'stress response'. (In several eukaryotic systems too, UV light can cause the induction of proteins which may be involved in repair including ones analogous to RecA [5], as well as other proteases of more general action [6–9].) It was decided to use the three treatments of UV irradiation, nalidixic acid and heat shock on the thermophilic cyanobacterium, *Phormidium laminosum* to identify proteins synthesised in response to DNA damage and to determine which may be directly associated with DNA repair (and possibly recombination) rather than stress responses. Identification of such proteins will be an important step towards elucidation of the mechanisms of repair and recombination in cyanobacteria.

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2. MATERIALS AND METHODS

2.1. *Strains and culture conditions*

P. laminosum strain OH-1-p clone 1 [10] was used throughout. Cells were grown in an orbital in-

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cubator at 45°C under continuous light in an atmosphere enriched with 5% CO_2. The culture medium was medium D of Castenholz [10], supplemented with $NaHCO_3$ (0.4 g/l).

2.2. *Irradiation and labelling conditions*

Mid-log phase cultures were used throughout and exposed to UV doses of 130–400 J/m^2 (254 nm radiation), giving a range of killing of 20–80% (Osborn, R.W., unpublished). Aliquots (10 ml) of irradiated or unirradiated cells were incubated for 1 h before addition of 150 μCi [^{35}S]methionine (Amersham International; 15 mCi/ml, 1200 Ci/mmol). The labelled cells were incubated for a further 2 h before harvesting by centrifugation. The pellet was resuspended in 1 ml wall digestion buffer (3 mg/ml lysozyme in 75 mM Tris-HCl, 20 mM EDTA, 100 mM NaCl, pH 8.0) and left at 20°C for 10 min. After this time the samples were centrifuged and the supernatant removed. The pellet was dissolved in 100 μl solubilising buffer (8% SDS, 20% glycerol, 0.01% bromophenol blue, 0.2 M Tris-HCl, pH 6.8).

2.3. *Nalidixic acid treatment*

Aliquots (10 ml) of mid-log phase cells were harvested by centrifugation and resuspended in 10 ml fresh medium D containing 50 μg/ml nalidixic acid (Sigma) and incubated as above for 0–90 min before labelling and harvesting as for UV-irradiated cells. Cells centrifuged and resuspended in fresh medium D without nalidixic acid were used as a control treatment.

2.4. *Heat shock*

Two forms of experiment were carried out, short and prolonged. Cells in 'short' shock experiments were incubated at 55°C for 10, 20 or 30 min. Cells were returned to the normal growth temperature and labelled and harvested as for UV-treated cells. In 'prolonged' shock experiments cells were grown at the elevated temperature for 1 h prior to addition of [^{35}S]methionine and labelling was carried out for 2 h at the elevated temperature before harvesting as for the other treatments. A temperature shift of 10°C has been shown to be more than sufficient to induce heat shock polypeptides in the cyanobacterium *Anacystis nidulans* [11].

In all experiments 'shocked' and 'control' cells received the same amount of visible light in order to avoid complications due to the possible synthesis of dark specific polypeptides observed in other cyanobacteria [12,13].

2.5. *Sample preparation*

Protein extracts in solubilising buffer were mixed 5:5:1 (by vol.) with fresh solubilising buffer and β-mercaptoethanol. Prior to electrophoresis samples were treated in one of three ways: (i) 1 h at 0°C; (ii) 30 min at 37°C; (iii) 3 min at 100°C. All samples were then centrifuged for 2 min to remove cell debris before electrophoresis.

2.6. *Electrophoresis and detection of polypeptides*

The protein supernatants were analysed by electrophoresis through 15% SDS-PAGE gels according to Laemmli and Favre [14], and proteins were detected by fluorography [15]. Gels were dried and placed against a sheet of preflashed X-ray film (Kodak X-Omat S) and exposed at −80°C for between 4 and 48 h before developing.

The molecular masses of polypeptides on SDS-PAGE gels were determined by comparison with ^{14}C-labelled proteins (Amersham) after co-electrophoresis.

3. RESULTS

3.1. *UV treatment*

Immediately following irradiation, incorporation of [^{35}S]methionine into newly synthesised polypeptides was low (not shown). A post-irradiation incubation period of 1 h was employed, prior to addition of label, to permit good detection of polypeptides. This preincubation would also allow time for the synthesis of mRNAs for any proteins induced by the UV treatment. Irradiation of cells with a UV dose of 130 J/m^2 was consistently found to induce or enhance specifically the synthesis of three polypeptides (fig.1) with apparent molecular masses of 43, 34 and 26 kDa. At higher UV doses the levels of polypeptide synthesis decreased, although at different rates. The effect on the level of synthesis of the three induced polypeptides was much less pronounced and following a UV dose of 400 J/m^2 the 26 kDa popypeptide accounted for approx. 70% of the label incorporated into polypeptides (not shown).

Three methods of sample preparation were used

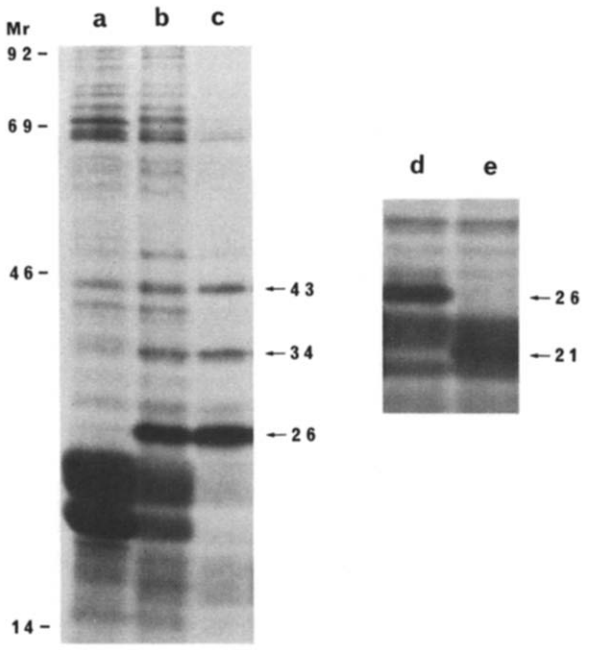

Fig.1. Fluorograph of total cell lysate of *P. laminosum*. Polypeptide profile of samples solubilised at 37°C for 30 min, (a) unirradiated cells; cells irradiated with (b) 130 J/m² or (c) 270 J/m² UV light. Lysate from UV-irradiated cells (270 J/m²) solubilised at (d) 37°C for 30 min or (e) 100°C for 3 min prior to SDS-PAGE. Enhanced/induced polypeptides are indicated (43, 34, 26, 21).

prior to loading of samples onto SDS-PAGE gels (section 2). In samples solubilised on ice for 1 h or at 37°C for 30 min the apparent molecular masses of the three polypeptides were as indicated above, but when solubilised at 100°C for 3 min the apparent molecular mass of the smallest polypeptide changed from 26 to 21 kDa (fig.1, lanes d,e). No other polypeptide, induced or constitutive, exhibited a similar reaction to this solubilising procedure. The decrease in apparent molecular mass of this polypeptide was not associated with the appearance of any low molecular mass polypeptide(s) which might account for the 5 kDa difference. The transition from the 26 to 21 kDa polypeptide was followed by solubilising samples for various times at 60°C in the presence or absence of β-mercaptoethanol. It was noted that the change from the 26 to 21 kDa form was much more rapid in the presence of β-mercaptoethanol than without it (not shown).

3.2. *Nalidixic acid*

The synthesis of three polypeptides was induced, or specifically enhanced, in cells after incubation with nalidixic acid. These three had apparent molecular masses closely similar to those of the polypeptides observed in UV-irradiated cells (fig.2). When samples were prepared for electrophoresis by heating to 100°C for 3 min the apparent molecular mass of the 26 kDa polypeptide changed to 21 kDa (not shown), providing good evidence that this polypeptide was the same as that induced in

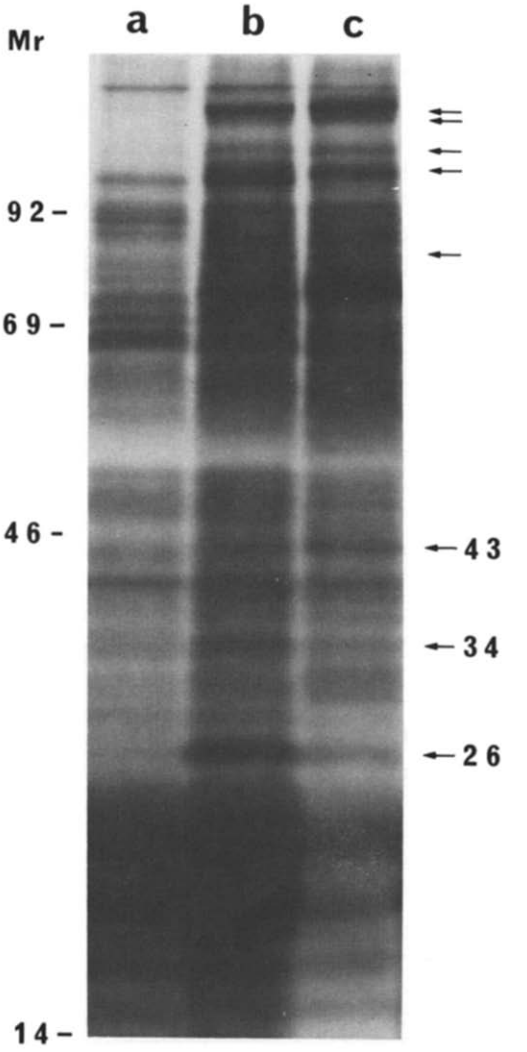

Fig.2. Fluorograph of total cell lysate of *P. laminosum*: (a) control cells, (b) cells incubated with nalidixic acid (50 μg/ml) for 30 min and (c) 90 min before labelling. Enhanced/induced polypeptides are indicated.

UV-treated cells. Several high molecular mass polypeptides (mostly >100 kDa) were also observed in some experiments but induction of these polypeptides was less consistent and they were not investigated in detail.

3.3. *Heat shock*

Cells exposed to an elevated temperature for a short period before labelling under normal growth conditions were observed to possess three induced, or specifically enhanced, polypeptides with apparent molecular masses of 89, 86 and 33 kDa (fig.3, lane a). Apart from this, the overall polypeptide synthesis profile in short heat shocked cells was broadly similar to that in unshocked cells.

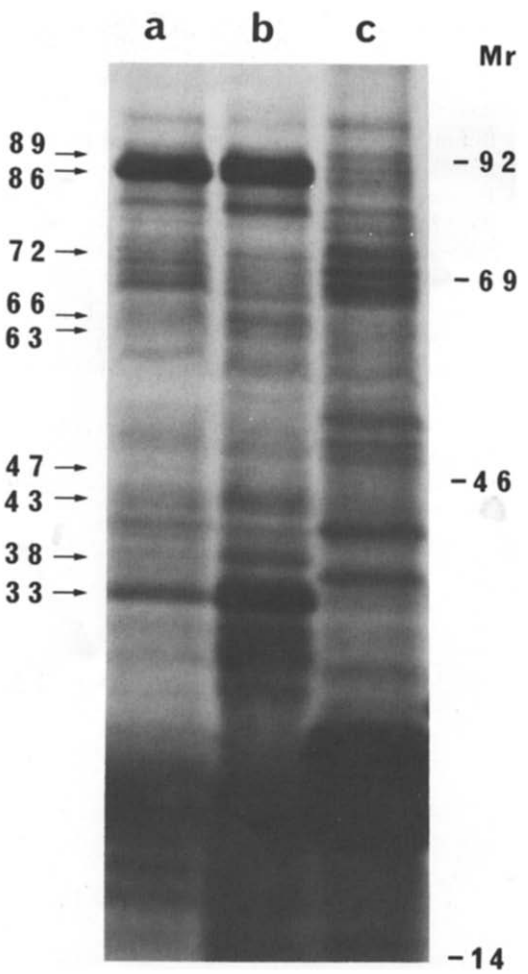

Fig.3. Fluorograph of total cell lysate of *P. laminosum*. Cells heat shocked at 55°C for (a) 30 min and (b) 3 h, (c) untreated control cells. Polypeptides enhanced/induced by heat shock are indicated.

Longer periods of heat shock, up to 3 h, with labelling at the elevated temperature, had a greater effect on the overall polypeptide synthesis profile and induced, or specifically enhanced, the synthesis of several additional polypeptides notably ones with apparent molecular masses of 72, 66, 63, 47, 43 and 38 kDa (fig.3, lane b). The 43 kDa polypeptide appeared to be the same as the 43 kDa polypeptide induced by UV and nalidixic acid.

4. DISCUSSION

The synthesis of three polypeptides has been found to be enhanced in *P. laminosum* following UV irradiation. The apparent molecular masses of two of these polypeptides were 43 and 34 kDa but that of the third polypeptide was dependent upon the method of sample preparation. If the sample was boiled for 3 min prior to electrophoresis the apparent molecular mass was 21 kDa but if solubilised at a lower temperature it was 26 kDa. The effect of β-mercaptoethanol on the mobility of this polypeptide in SDS-PAGE systems suggests that when solubilised at 37°C or below certain disulphide bridges remain intact thereby reducing the quantity of SDS bound and in turn reducing the mobility of the polypeptide upon electrophoresis.

Following exposure to nalidixic acid the synthesis of a number of polypeptides was enhanced and three of these had closely similar molecular masses to those induced by UV. The 26 kDa polypeptide exhibited the same dependence upon sample preparation as that in UV-treated cells. Thus, it is clear that this protein, at least, is induced by both UV light and nalidixic acid. It is highly likely that the 43 and 34 kDa polypeptides induced by nalidixic acid treatment are also identical to those induced by UV and this possibility is currently under examination using 2D gel electrophoresis.

Short heat shock was found to enhance the synthesis of three polypeptides (89, 86, 33 kDa). More prolonged treatment enhanced the synthesis of a number of additional polypeptides, one of which (43 kDa) was also apparently enhanced in UV and nalidixic acid-treated cells. Polypeptides with a wide range of molecular masses have been shown to be induced by heat shock in the cyanobacterium *A. nidulans* [11].

It is therefore clear that the 26/21 kDa polypeptide induced after UV irradiation is also induced by treatment with nalidixic acid, but not in response to heat shock. This suggests that this polypeptide may be involved in DNA repair (and possibly, therefore, recombination in an SOS-type response). The same may also be true of the 34 kDa polypeptide induced after exposure to UV or nalidixic acid, although the molecular masses of these polypeptides are not within the range reported for proteins analogous to RecA [3,16–18], including that reported from the cyanobacterium *Anabaena variabilis* [19]. The 43 kDa polypeptide may be part of a wider stress response. The fact that the polypeptides, particularly the 26/21 kDa one, represent such a large proportion of total protein synthesis in irradiated cells should facilitate their purification and characterisation. The possible functions of all three polypeptides are currently being investigated.

ACKNOWLEDGEMENT

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